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The microflora in sand from dairy bed-cubicles

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Introduction

Infection with environmental bacteria such as *E. coli* and *Klebsiella* spp. is a common cause of mastitis, milk loss, and culling on dairy farms. Especially *Klebsiella* spp. mastitis can cause a considerable and often sustained decrease in milk production. In some cases an average loss of 7.6 kg/day immidiatly after infection and 5 kg/day in subsequent months has been reported (Grohn *et al.,* 2004). Control of *Klebsiella* spp. and other environmental mastitis types is largely based on prevention of exposure of the udder to the pathogen. There has been less progress on control of mastitis caused by environmental pathogens compared with contagious pathogens (Smith and Hogan, 2001). Since many dairy farmers bed stalls with sand at least once a week, monitoring the dynamics of the bacterial populations in sand over time in the stalls would provide valuable information on these pathogens.

Methods

Nineteen herds with sand bedded free stalls were enrolled in the study based on a history of infections of predominantly *E. coli* and *Klebsiella* spp. From each herd, sand was sampled from all sections with lactating cows. From each section 5 stalls were sampled systematically to ensure inclusion of all areas of each section, and all parts of each stall. Only stalls with no visual feces or urine and with no pyramidal piling of the sand were sampled. Only one of the 19 farms had implemented recycling of sand. All samples were collected wearing gloves. In the laboratory, the samples were examined with bacteriological culture including MALDI-TOF MS, 16S rDNA amplicon sequencing, and a commercial qPCR (DNA diagnostic). From 16 of the participating farms, quarter milk samples were collected from cows with a new infection at the latest DHI recording before sampling. This produced a total of 217 quarter milk samples from 132 cows. The milk samples were examined by bacteriological culture and MALDI-TOF MS.

Results and Discussion

Preliminary comparison was carried out on the samples from the toplayer of the sand in the tails end of the stalls, as these were expected to present the highest numbers of coliforms. In bacteriology, the quantum of live bacteria was substantial, and could

therefore not always be enumerated even at -6 dilution a total-CFU. However, only few coliforms were found and samples from only one farm was found positive for live *Klebsiella* spp. With 16S rDNA sequencing, a wide spectrum of bacteria was present in the sand, but the class encompassing enterobacteriaceae was not among the most frequently present. The 16S rDNA sequences furthermore showed a clear clustering by farms . qPCR analyses showed that DNA from *E. coli* and/or *Klebsiella* spp. was present in samples from 6 out of the 19 farms. However, the qPCR also showed that differences in the DNA-extraction protocol could affect the results considerably. Comparison of the bacteriological culture of sand and milk showed no apparent coherence.

Altogether, the load of *E. coli* and/or *Klebsiella* spp. in the sand was lower than expected based on studies from other studies (KILDER). A bias in the bacteriological culture approach is the high amount of bacteria in the sand, requiring high dilutions before culture. Accordingly, some false negative culture results might occur. Nevertheless, when comparing the three analyses it seems that they agree on an over-all low amount of *E. coli* and/or *Klebsiella* spp. in the sand.

Conclusions

These preliminary data are currently being further investigated by deeper 16S rDNA sequencing and a re-run of the qPCR analysis with another DNA extraction protocol. Nevertheless, based on the apparent agreement of the three analyses, a large quantum of *E. coli* and/or *Klebsiella* spp. is not expected to be found.

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References

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